REMARKS

Claims 1-50 are in the case.

Claims 5, 9, 11, 14, 16, 18, 20, 22, 24, 26, 28-34, 36 and 41-47 are canceled.

Claims 1-4, 6-8, 10, 12, 13, 15, 17, 19, 21, 23, 25, 27, 35, 37-40, and 48-50 are under consideration in response to the current office action.

Claims 1,3, 7, 8, 12, 39, 39 and 49 and have been amended to more clearly define Applicants' invention.

Claims 2, 4 and 6 have been additionally canceled by this Response and Amendment.

The claims have been made subject to a requirement to restrict and an election of species. Applicants have agreed to an election of species, consisting of: *Methylomonas* as a host cell, Astaxanthin as a carotenoid substrate, and a set of enzymes required for the production of Astaxanthin consisting of SEQ ID NO:.26 encoding geranylgeranyl pyrophosphate (GGPP) synthase; SEQ ID NO:34, encoding phytoene synthase; SEQ ID NO:32 encoding phytoene desaturase; SEQ ID NO:30 encoding lycopene cyclase; SEQ ID NO:36 encoding β-carotene hydroxylase; and SEQ ID NO:38 encoding β-carotene ketolase.

All claims stand rejected variously under 35 USC § 112, 102 and 103.

No new matter has been added.

Applicants submit herewith a declaration under Rule 132 in support of the assertions made herein.

Objections to the Specification

The specification is objected to under 35 USC § 112, first paragraph, and claims 12 and 38 have been rejected to as the specification is considered non-enabling for the claimed and deposited microorganism. Specifically the deposited microorganism does not appear to be readily available to the public.

While not intending to agree with the Examiner's reasoning concerning whether the novel organism is essential to the claimed invention, Applicants note that a declaration of biological deposit was submitted with the present application at the time of filing and submit that this declaration is sufficient to overcome the present objections. Applicants submit herewith a copy of said declaration as well as the return receipt post card indicating initial receipt by the Patent Office.

Claim Rejections – 35 USC § 112, First Paragraph

Claims 1-4, 6, 7, 10, 13, 15, 17, 19, 21, 23, 25, 27, 35, 39, 40, and 48-50 have been rejected under 35 USC § 112, first paragraph. The Examiner is of the opinion that the disclosure is enabling only for claims limited to a method of making carotenoids utilizing a transformed methylomonas with genes required for the biosynthesis of carotenoids, thus the claims are considered broader than the enablement provided by the disclosure with regard to all possible

organisms, which are able to metabolize C1 compounds such as methane, methanol, formaldehydes, formic acid, methylated amines, and methylated thiols. Applicants respectfully traverse.

With respect to enablement for substrates broader than methane: Applicants note that the exemplified *Methylomonas* strain is a methanotrophic bacterial host capable of growing on methane and/or methanol. Support for the use of methanol as a substrate is found in the specification (see page 11, line 22, in the definition of high growth methanotrophic bacterial strain; and page 24, the discussion beginning at line 13, where methanol is a substrate used in standard isolation procedures.).

The exemplified *Methylomonas* strain (*Methylomonas* 16a) is a Type I bacterial methanotroph. As stated on page 3, lines 24-31, methanotrophs are a subclass of methylotrophs, capable of using a variety of C1 compounds as a sole carbon source. Consequently, the Applicants have amended the claims, limiting the scope of the claimed invention to methylotrophic bacteria capable of using methane and/or methanol as the C1 carbon source.

The Examiner states in the present office action that "The specification teaches special characteristics of methylomonas 16a, which makes it " ...uniquely suitable for the claimed method. Thus, searching for a suitable organism, which metabolizes C1 compounds having similar characteristics to methylomonas 16a is well outside the realm of routine experimentation and predictability in the art of success is extremely low ." The Applicants respectfully disagree.

The claims as amended describe a method for the production of a carotenoid compound using a transformed methylotrophic bacterial strain comprised of isopentenyl pyrophosphate and at least one isolated nucleic acid fragment encoding an enzyme in the carotenoid biosynthetic pathway, wherein the methylotrophic bacterial strain is capable of growing on methane and/or methanol. *Methylomonas* 16a is exemplified because it is a high growth methanotrophic strain. However, the desirable growth characteristics of this strain should not limit the scope of the claimed invention. The ability to convert methane and/or methanol into central carbon metabolism intermediates, specifically glyceraldehyde-3-phosphate and pyruvate, is found in essentially all methylotrophic bacteria (Figure 2). Additionally, the isoprenoid biosynthesis pathway is commonly found in many microorganisms, producing isopentenyl pyrophosphate (IPP) and farnesyl pyrophosphate (FPP). IPP and FPP are typically used as building blocks to make many compounds commonly found in many microorganisms such as isoprene, ubiquinones [derived from "ubiquitous"], hopanoids, phytols, terpenes, sterols, and polyprenols, to name a few. However, the carotenoid biosynthesis pathway, which typically uses IPP as a starting substrate (Figure 1) is not found in many microorganisms. However, recombinant expression carotenoid biosynthesis genes have been reported in many (frequently non-naturally pigmented) microorganisms such as *E. coli*, *Candida utilis*, *Phaffia rhodozyma*, *Zymomonas mobilis*, and *Saccharomyces cerevisiae* (see description starting on page 2, line 27 through page 3, line 15). Additionally, the *Pantoea stewartii crt* gene cluster expressed in *Methylomonas* 16a (see

Example 9, pages 69-71) has been recombinantly expressed by the Applicants in *E. coli* (data supplied in presently attached Rule 132 declaration). Expression of carotenoid biosynthesis genes in highly divergent hosts would indicate to one of skill in the art that expression of a carotenoid biosynthesis gene would reasonably be expected to work in a host cell more closely related to the exemplified host cell (i.e. methylotrophs and/or other methanotrophs).

In view of the foregoing Applicants submit that the specification is enabling for claims limited to the methylotrophs using substrates selected from methane and methanol.

Claim Rejections – 35 USC § 112, Second Paragraph

Claims 1-4, 6-8, 10, 12, 13, 15, 17, 19, 21, 23, 25, 27, 35, 37, 38-40, and 48-50 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter.

Rejection A. It is the Examiner's opinion that the phrase "suitable levels of isopentenyl pyrophosphate" in claims 1, 39, and 49 render the claims indefinite because the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. Neither the claim or the specification define the suitable levels of isopentenyl pyrophosphate that is required to carry our the claimed method; one of ordinary skill in the art would not know said suitable levels.

The Claims have been amended remove the reference to "suitable levels". The presence of isopentenyl pyrophosphate is clearly supported in the specification (see Figure 1 and throughout the specification).

Rejection B. The Examiner argues that the phrase "C1 carbon substrate.... Methylated amines, methylated thiols" in claim 2 render the claims indefinite and confusing because the resulting claim does not clearly set forth the metes and bounds of the patent protection desired. The Examiner is of the opinion the definition stated on page 11, lines 4-7, is repugnant to one of ordinary skill in the art.

Amendments to the Claims have rendered this rejection moot.

Rejection C. Claim 38 is rejected as it recites the limitation "wherein said methanotrophic bacteria" in claim 1. There is insufficient antecedent basis for this limitation in the claim.

The Claims have been amended to overcome this rejection.

Rejection D. Claim 3, 4, 6-8, 10, 12, 13, 15, 17, 19, 21, 23, 25, 27, 35, 37, 40, 48, and 50 are included in the rejection because they are dependent on rejected claims and do not correct deficiencies of the claim from which they depend.

The claims have been amended to overcome this rejection.

Claim Rejections Under 35 USC § 102(b)

Claims 1, 2, 13, and 48 are rejected under 35 U.S.C. 102(b) as being anticipated by U. S. Patent 5,545,816 [Ausich et al., ('816)]. The '816 patent teaches the transformation of plant cells

with nucleic acid sequences encoding geranylgeranyl pyrophosphate (GGPP) synthase and phytoene synthase and a method of producing phytoene from the transgenic plant. The use of carbon dioxide by the transgenic plant is considered to be a C1 compound.
A valid rejection under 35 USC § 102(b) requires that all the elements of the claimed invention be described in the cited reference. The claims have been amended limited to methylotrophic hosts capable of metabolizing/assimilating methanol and/or methane. As such the '816 patent does not teach all the elements of the claimed invention.

In view of the foregoing Applicants respectfully request removal of this rejection.

Claim Rejections Under 35 USC § 103(a)

Claims 39, 40, and 49 are rejected under 35 U.S.C. 103(a) as being unpatentable over '816 patent in view of Albrecht et al. (*Biotech Lett* (1991) 21:791-795), (hereinafter "Albrecht"). In making this rejection the Examiner notes that the application names joint inventors and presumes that the subject matter of the various claims was commonly owned at the time any invention were made. The Examiner's presumption is correct.

The teachings of the '816 patent are described above. Albrecht et al. teach the transformation of *E. coli* producing β-carotene with nucleic acid required in the biosynthesis of prenyl pyrophosphates lead to an increase in the production of β-carotene.

It is the Examiner's opinion that the skilled person would be motivated to combine the teachings of Albrecht demonstrating that overexpression of upper carotenoid pathway genes leads to increased levels of carotenoids in E. coli with the teachings of plant transformation with geranylgeranyl pyrophosphate (GGPP) synthase to derive the present invention. Applicants respectfully traverse.

It is axiomatic that for a rejection under 35 USC § 103 to be valid three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. (MPEP 2143)

Applicants submit that the references cited in the present rejection do meet the test outlined above. The cited references relate to the transformation of plants and *E. coli* for the production of carotenoids. The present invention relates to the transformation of methylotrophic bacteria, growing on either methane or methanol for the production of carotenoids.

With respect to motivation, the skilled person would not be motivated to look to methylotrophs as a production host based on the teachings of the cited references. As compared with the genetic systems of methylotrophs the systems of *E. coli* and plants are well characterized and methods and techniques for genetic transformation are well established.

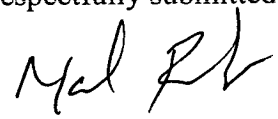
Transformation and expression of genes in most methylotrophs is still a difficult and time consuming process relying on bacterial conjugation (see example 9 of the present application). The skilled person would not find the teachings of either reference suggestive of the use of methylotrophs to accomplish the present invention.

With respect to the issue of reasonable expectation of success: Again, as discussed above, methylotrophic bacteria are genetically divergent from either plants or *E. Coli* and one of skill in the art would not reasonably expect that expression of genes in either of these hosts would necessarily lead to success in a methylotroph.

With respect to the presence of all the claimed elements in the combined references: It is clear that the combination of '816 and Albrecht do not teach or suggest the presence of methylotrophic bacteria or the use of the substrates methane or methanol. Thus not all the limitations of the claimed invention can be found in the combined references.

In view of the foregoing Applicants respectfully request removal of all rejections and reconsideration of the claims as amended.

Respectfully submitted,



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